Molecular phylogeny, biogeographic history, and evolution of cave-dwelling taxa in the European harvestman genus *Ischyropsalis* (Opiliones: Dyspnoi)

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Abstract. We estimated a multigenic molecular phylogeny and reconstructed biogeographic history for the European harvestman genus *Ischyropsalis* C.L. Koch 1839 (Dyspnoi). To reconstruct historical biogeographic patterns we conducted an algorithmic VIP analysis which revealed patterns consistent with a vicariance-dominated history. The existing morphology-based systematic framework for *Ischyropsalis* is mostly inconsistent with molecular phylogenetic results, and a new informal system is established that recognizes three main clades and several sub-clades. Species-level analyses revealed two non-monophyletic species (*I. pyrenaea* Simon 1872 and *I. luteipes* Simon 1872); subspecies of *I. pyrenaea* are distant relatives, and are formally elevated to species (*I. pyrenaea pyrenaea* to *I. pyrenaea* and *I. pyrenaea alpinula* to *I. alpinula*). A preference for cryophilic microhabitats has favored the diversification of high-altitude and cave-dwelling *Ischyropsalis* species; molecular phylogenetic data suggest that cave-dwelling species have evolved multiple times independently.

Keywords: Species tree, multilocus phylogeny, biogeographic modelling, convergence, ecological specialization, cave evolution

Modern biogeographic studies of harvestmen have revealed an interesting mixture of evidence for extremely limited dispersal (e.g., Thomas & Hedin 2008; Derkarabetian et al. 2011; Boyer & Reuter 2012), combined with evidence for occasional long-distance dispersal (e.g., Shultz & Regier 2009; Sharma & Giribet 2012; Schönhofer et al. 2013). As such, harvestmen are compelling systems for biogeographic research, but as expected many harvestmen taxa remain to be investigated using modern phylogenetic and biogeographic methods. Within the European fauna, members of the genus *Ischyropsalis* C.L. Koch 1839 are among the most charismatic harvestmen, with relatively large body sizes and massively enlarged chelicerae used to catch prey (Fig. 1). Ecologically, members of this genus are often rare and found in difficult-to-access habitats, including several obligate cave-dwelling species.

Ischyropsalis species limits have been investigated in the context of the biological species concept (Mayr 1942), rarely explicitly applied within arachnids. Martens (1969a) observed *Ischyropsalis* males offering females a secretion from the basal cheliceral segment during courtship and copulation, and demonstrated an association between the secretion offering and subsequent contact between this secretion area and the female mouthparts (Martens 1969b). The male cheliceral secretion area includes bristle fields (Fig. 1) that are connected by minute pores to massive glands (Martens & Schawaller 1977). Martens (1969b) made this cheliceral secretion area and the resulting associated male/female interaction a fundamental character to delineate *Ischyropsalis* species, and also found corresponding male genital characteristics. The presence of such character combinations has also been adopted for species delimitation and higher-level classification in other Opiliones, for example in the Nemastomatidae (Schönhofer & Martens 2012), Dicranolasmatidae (Gruber 1998) and Sabaconidae (Martens 1972; Suzuki 1974).

Studies of *Ischryopsalis* classification began with Hadži (1931), who delineated a single *Ischyropsalis* subgenus, *Odontopalpa,* based on apophysis characters on the male palpal patella (Fig. 1). Originally containing three species, two species were synonymised (Martens 1969b) with *I. kollari* C.L. Koch 1839 (*I. bosnica* Roewer 1914, *I. triglavenis* Hadži 1931), the type species of *Ischyropsalis.* As it was not considered in *Odontopalpa,* the subgenus then became invalid. Hadži originally also placed *I. dentipalpis* Canestrini 1872 and later *I. ravasinii* Hadži 1942 within *Odontopalpa* but had no males to confirm the male-specific characters of this subgenus for the latter. Roewer (1950) described numerous new *Ischyropsalis* species, but was suspected to have separated specimens of common species to describe new species. Martens (1969b) detected these manipulations, later substantiated by v. Helversen and Martens (1972), and synonymized 33 of 34 of Roewer's species.

Morphology-based phylogenetic hypotheses for *Ischyropsalis* were published nearly simultaneously by Martens (1969b) and Dresco (1970). Dresco drew his conclusions from the Paris Museum collection, containing mostly Iberian *Ischyropsalis,* while he had limited access to and knowledge of the remaining European fauna. Martens (1969b) considered most currently-accepted species, and provided an extensive revision based on characters newly developed for *Ischyropsalis* systematics. The systems of Martens and Dresco only agree in the isolated position of *I. hellwigii* (Panzer 1794) while other hypothesised groupings show little congruence (Fig. 2). Authors after 1970 did not comment on the phylogenetic structure of the genus, but added new species or further clarified species synonymies. Martens (1978) refined his system for some Alpine species. Luque (1991, 1992) and Prieto (1990a, b) described the Spanish fauna in more detail. Most recently, Schönhofer and Martens (2010a) and Luque and Labrada (2012) added two new species and discussed regional species diversity in the
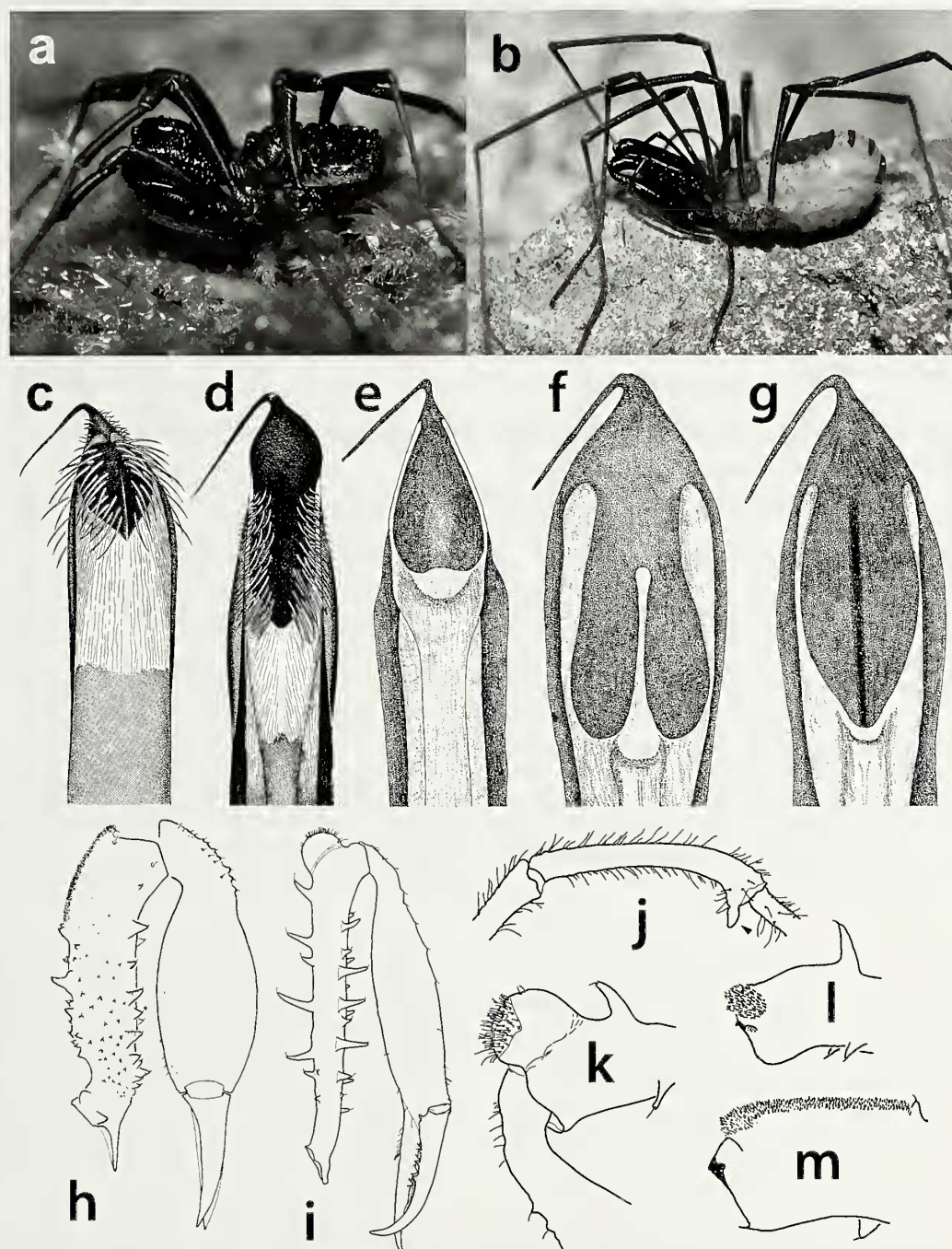
Figure 1.—*Ischyropsalis* habitus and morphological features. a) *I. hellwigii hellwigii* male habitus, Austria, Soboth; b) *I. pyrenaea pyrenaea* female habitus, France, Grotte de l'Estelas; c) distal part of penis, *I. pyrenaea alpinula* (Martens 1978: Fig. 381); d) distal part of penis, *I. dentipalpis* (as *I. helvetica*; from Martens 1978: Fig. 382); e–g) different types of glans sclerites (groups *sensu* Martens, 1969b; Fig. 32): e) *I. hellwigii* group, f) *I. kollari* group, g) *I. dentipalpis* group; h, i) male cheliceral armament, h) *I. hellwigii hellwigii* (Martens 1978: Fig. 289), i) *I. dentipalpis* (Schönhofer & Martens 2010a); j) apophysis on palpal patella of male *I. dentipalpis* (Schönhofer & Martens 2010a; Fig. 6); k–m) distal part of male chelicerae (medial view), apophyses and cheliceral secretion-extruding-field indicated by bristlefield: k) *I. lithoclasica* (Schönhofer & Martens 2010a; Fig. 13), l) *I. adamii*, m) *I. hellwigii* (both Martens 1969b; Fig. 22).

Western Alps and the Cantabrian Mountains, respectively. Twenty-two species-level taxa are currently recognized in the genus (Schönhofer 2013a).

Based on the revision of Martens (1969b), distributional patterns in *Ischyropsalis* suggest a complex biogeographic history. The three species groups of Martens (1969b) are widespread and overlap in distribution, while species within groups are strictly allopatric. This broad-scale phylogenetic structure and the proposed species inter-relationships imply repeated dispersal and isolation between the major mountain systems in Western and Central Europe. On the other hand, individual *Ischyropsalis* species have generally narrow distributions, and at least nine species are obligate cave endemics. Cave-dwelling species are unlikely to disperse over large
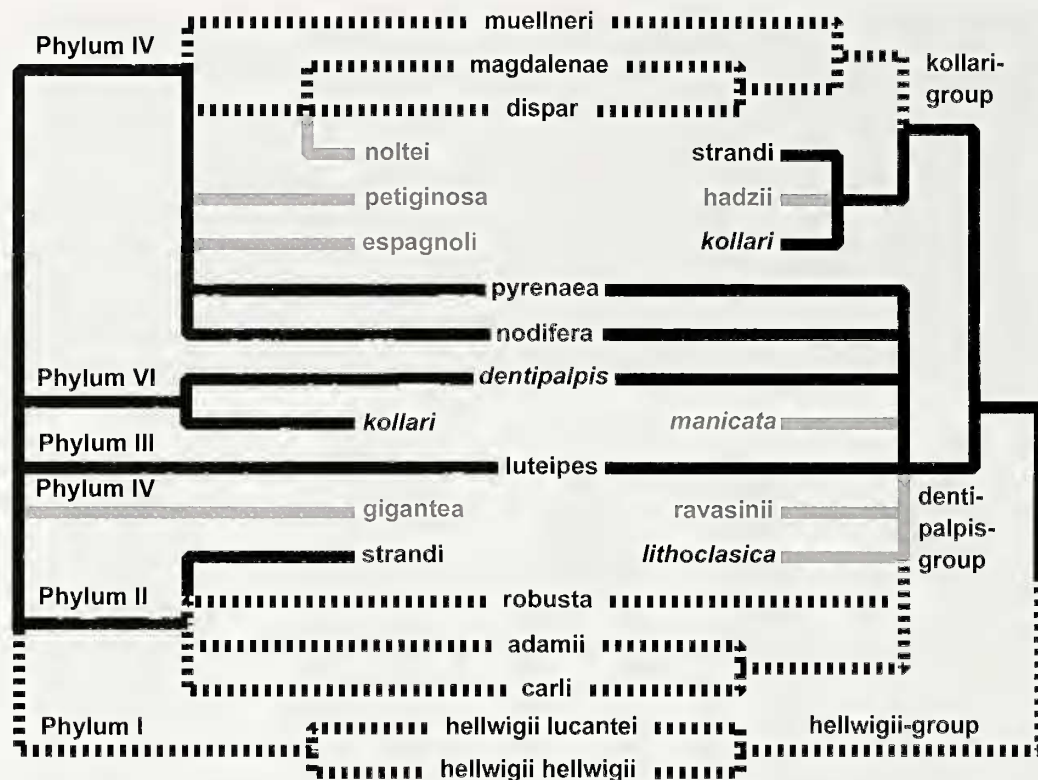
Figure 2.—Morphology-based phylogenetic hypotheses for *Ischyropsalis* according to Dresco (1970, left) and Martens (1969b, right). Some synonymies / misinterpretations used by Dresco were replaced for ease of comparison (we use *I. muellneri* for *I. hellwigii*, *I. hellwigii hellwigii* for *I. taunica*, *I. adamii* for *I. apuanus*, *I. kollari* for *I. trigalvensis* and *I. hellwigii lucautei* for *I. superba*). Phylogenetic lineages are compared and matched if their taxa composition shows at least two matches agreed upon by both authors; matching taxa are indicated on dashed branches; taxa corresponding to alternative lineages are indicated on black branches; taxa considered by only one author are designated in grey. Non-matching species are separated by space between the species names. Species of subgenus *Odontopalpa* sensu Hadži (1931) shown in italics.

distances, and may have originated from widespread epigean species (Porter 2007).

We present here the first molecular phylogenetic analysis of *Ischyropsalis* to investigate the validity of alternative higher-level classification schemes as well as multiple species and subspecies hypotheses (Martens 1969b; Dresco 1970). This molecular phylogenetic framework also allows us to address the phylogenetic value of currently used morphological characters—because of their relatively simple organization and structure. Martens (1969b) suggested the possibility of morphological convergence in male genitalia of *Ischyropsalis*. The distribution of all *Ischyropsalis* taxa is reconstructed based upon literature, museum collections and personal collection data, and these data are used in algorithmic analyses to reconstruct *Ischyropsalis* biogeographic history. Finally, molecular phylogenetic results provide insight into whether cave-dwelling taxa originate from a single ancestor, or have evolved independently multiple times.

METHODS

Sampling.—*Ischyropsalis* specimens were collected by hand, mainly during our own field excursions. Some specimens were also generously donated by colleagues (see Acknowledgments). Specimens were preserved in 100% EtOH, with the majority being collected in 80% and transferred soon thereafter. Morphological identifications were based on

Martens (1969b, 1978), Luque (1991, 1992) and Schönhofer and Martens (2010a), or provided by C. Prieto for Spanish samples. A few specimens were raised to adulthood in captivity to allow morphological species identification. We included as many described species as possible, and conducted intraspecific sampling to include geographically and morphologically distinct lineages. Voucher specimen details and repository information are provided in the Appendix. Voucher specimens (with associated collection numbers) are deposited in the collections of J. Martens (CJM, Mainz, Germany), C. Prieto (CCP, Bilbao, Spain), A. Schönhofer (AXLS, Mainz, Germany), C. Vernesi (FEM: Centre for Research and Innovation—Fondazione Edmund Mach, San Michele all'Adige, Italy) and the San Diego State University Terrestrial Arthropod collection (OP).

Molecular methods and analyses.—Genomic DNA was extracted from tissue using the Qiagen DNeasy kit (Valencia, CA, USA). The polymerase chain reaction (PCR) was used to amplify the following gene fragments: 28S rRNA (28S), using the primers ZX1elong (Schönhofer et al. 2013), ZX1, and ZR2 (Mallatt & Sullivan 1998); mitochondrial Cytochrome Oxidase 1 (CO1), using C1-J-1718SPIDERA and C1-N-2776SPIDER (Vink et al. 2005); and nuclear Elongation Factor 1-alpha (EF1α), using OP2BSAB and OPRC4 (Hedin et al. 2010). PCR protocols followed Hedin et al. (2010) for EF1α, Thomas and Hedin (2008) for CO1, and Hedin and Thomas (2010) for 28S, the latter using an annealing temperature of 56°C.

Amplicons were purified on Millipore plates and directly Sanger sequenced at Macrogen USA in both directions. SEQUENCHER v4.5 was used to assemble and edit sequence contigs, with ambiguous sites scored using standard ambiguity codes.

CO1 and EF1α exon sequences were aligned manually in Mega 4.0 (Tamura et al. 2007) using amino acid translation, while 28S and EF1α intron sequences were aligned with MAFFT vers. 6 (http://mafft.cbrc.jp/alignment/software/), using the Q-INS-i strategy as recommended by Katoh and Toh (2008). To account for alignment uncertainty in the 28S and the EF1α intron data, outgroup genera were removed and the data were realigned using the same strategies as above. Models of DNA sequence evolution were evaluated using jModelTest 0.1.1 (Posada 2008) under three substitution schemes (JC, HKY, GTR) on a fixed BIONJ tree, allowing for unequal base frequencies and among-site rate variation. Final model selection was based on the Akaike Information Criterion (AIC) and individual models were applied to respective partitions in all downstream phylogenetic analyses.

Bayesian inference using MrBayes v3.2.0 (Huelsenbeck & Ronquist 2001; Ronquist et al. 2011) was applied to single genes as well as concatenated datasets. DNA sequences were partitioned by gene, EF1α by exon and intron, and CO1 and EF1α exons by codon position. Bayesian analyses were run for 5 million generations, where in all cases the standard deviation of split frequencies had dropped below 0.01 and standard convergence diagnostics were satisfied for all sampled parameters (ESS >200, PSRF = 1.00; Ronquist et al. 2011). Analyses were repeated to further check for convergence. The first 40% of trees were discarded as burn-in, with remaining trees used to reconstruct a maximum clade credibility tree. Split frequencies were interpreted as posterior probabilities (pp) of clades. A maximum likelihood (ML) concatenated tree using the partitioning scheme outlined above was reconstructed with the raxmlGUI (Stamatakis 2006; Silvestro & Michalak 2012), with support assessed using 1000 rapid bootstrap replications.

As an alternative to concatenation, a species tree was reconstructed using the multispecies coalescent model implemented in *BEAST (Heled & Drummond 2010; Drummond et al. 2012). Models of molecular evolution suggested by jModelTest were implemented, with codon partitioning applied to CO1 and EF1α exons ((1+2), 3). Priors were set to gamma if modified, otherwise default priors were used. Analyses were run until ESS values exceeded 200 for most priors, which was after 200,000,000 generations. Analyses were replicated twice and checked for convergence using Tracer v1.5 (Rambaut & Drummond 2007).

Species trees were estimated using two different sets of operational taxonomic units (OTUs). The first set included only described and accepted species and subspecies (= conservative species set), while the second set was extended to include additional genetic lineages within the species *I. adamii* Canestrini 1873, *I. dentipalpis* and *I. luteipes* Simon 1872 recovered in concatenated analyses (= diversified species set). Preference for one of these OTU settings was evaluated by comparing marginal likelihoods using Bayes Factor analysis (Grummer et al. 2014), as implemented in Tracer v1.5 (Rambaut & Drummond 2007).

Biogeographic analysis.—We compiled geographic distribution data for all known *Ischyropsalis* taxa. A total of 1,360 distribution records were extracted from literature, museum and personal collections, of which 1,220 could be georeferenced at a minimum accuracy of two digital degrees. Over 1,100 coordinates matched species and geographic lineages in our dataset and were used for biogeographic reconstruction. An interactive map with all referenced localities is available online (Schönhofer 2013b).

Applying the extant distribution of species and the species tree derived from *BEAST analyses, the Vicariance Inference Program (VIP, Arias 2010; Arias et al. 2011) was used to infer the distribution of clades and distributional barriers. This program reconstructs the probability of distributional barriers using parsimony. For area reconstruction in VIP the grid was set to 0.3 without fill. To account for misidentifications, a maximum area overlap of 5% was allowed between species. A heuristic search was conducted using 1000 iterations, setting full sector search and otherwise using default settings.

RESULTS

Molecular phylogeny.—A total of 39 *Ischyropsalis* specimens were included in molecular phylogenetic analyses, with samples available for all described subspecies, and 18 of 22 currently described species. Previously published sequences were included from GenBank for *I. luteipes*, *I. robusta* Simon 1872 and *I. nodifera* Simon 1879 (Shultz & Regier 2001; Schönhofer & Martens 2010b; Giribet et al. 2010; see Appendix). Outgroup sequences for the sister genera *Ceratolasma* Goodnight & Goodnight 1942 and *Acuclavella* Shear 1986 were obtained from Richart & Hedin (2013).

The protein alignments of CO1 and the EF1α exon contained no gaps and no stop codons. We note that although EF1α paralogs have been detected in mite harvestmen (Clouse et al. 2013), we found no evidence for paralogs in *Ischyropsalis*. PCR products directly Sanger sequenced showed no evidence for multiple overlapping sequences as would be expected if paralogs were common. However, this result is potentially biased by the PCR primers that we utilized. As such, we also searched for EF1α paralogs in the transcriptomes of other ischryopsalidoid taxa (Hedin et al. 2012; unpublished data). Specifically, we blasted *Ischyropsalis* EF1α sequences (*I. carli* Lessert 1905, AXLS 145) against the transcriptomes of *Sabacon* Simon 1879, *Acuclavella* and *Hesperonemastoma* Gruber 1970 (tblastx, e value 1e-20) in Geneious 7.0.6. These searches recovered a single EF1α sequence for each of these ischryopsalidoid taxa, suggesting a lack of paralogy in this clade. We emphasize that Opiliones is an old taxon with ancient internal divergences (Hedin et al. 2012), and that gene duplication in one lineage does not imply similar molecular evolutionary patterns in other, distantly related taxa. Similar dynamics of EF1α paralogy gain and loss are observed, for example, in insects (Djernaes & Damgaard 2006).

Models of sequence evolution were selected as follows: 28SrRNA: GTR+I+G; COI codon position 1: SYM+G; COI codon position 2: F81+G; COI codon position 3: GTR+G; EF1α codon position 1: HKY+I+G; EF1α codon position 2: HKY; EF1α codon position 3: GTR+G; EF1α intron: HKY+G. In *BEAST settings were modified for EF1α-exon to SYM+I+G (in *BEAST set to TN93+I+G, base frequencies

equal) and for COI to GTR+I+G (same in *BEAST), both separated by codon.

Ischyropsalis is clearly monophyletic with respect to the North American *Acuclavella* and *Ceratolasma*, allowing removal of these outgroups for further reconstructions. Results of the concatenated Bayesian and ML analyses, both with and without outgroups, were generally congruent (Fig. 3), with the few topological differences observed at weakly supported nodes. Alternative topologies recovered different arrangements between *Ischyropsalis lithoclasica* Schönhofer & Martens 2010a, *I. kollari* and *I. ravasinii*, and grouped *I. navarrensis* Roewer 1950 with *I. robusta* in the ML analyses. The same was true for the position of terminals within the two *I. luteipes* clades and *I. carli* (Fig. 3).

Bayesian and ML molecular phylogenetic results indicate that *Ischyropsalis* is divided into three primary clades, defined here for further discussion: 1) the *I. hellwigii* group (comprising the two described subspecies), 2) the *I. manicata* group (including *I. adamii*, *I. carli*, *I. manicata* L. Koch 1869 and *I. pyrenaea alpinula* Martens 1978), and 3) the Iberian-Alpine group, including all remaining sampled species. The composition of these groups is further detailed in the Discussion. Molecular phylogenetic support for these clades is strong (e.g., Bayesian posterior probability values > 0.95), although slightly lower for the *I. manicata* group, which includes several species that are missing data for multiple genes (Appendix).

Bayesian and ML concatenated analyses (Fig. 3) recovered most of the currently accepted species as well-supported lineages, with some exceptions. The subspecies of *Ischyropsalis pyrenaea* are not recovered as monophyletic, but instead fall into different higher-level groups. *Ischyropsalis luteipes* was found to be paraphyletic with *I. pyrenaea pyrenaea* nested within *I. luteipes*. Intraspecific genetic divergences were generally low, except for *I. adamii* and *I. dentipalpis*, both including divergent genetic lineages. Distinct lineages within the non-monophyletic or internally-divergent taxa were defined as distinct OTUs in the *BEAST analysis of the diversified species set, and all received strong support (Fig. 4). However, the Bayes Factor analysis did not conclusively favor one species-set hypothesis over the alternative hypothesis (In Bayes Factor: 3.635).

Biogeographic analysis.—VIP results are visualised in Figs. 5 and 6, including distributions of all higher clades (Fig. 5) and the Iberian-Alpine-group (Fig. 6). VIP recognized thirteen barriers corresponding to phylogenetic splits. Barriers were found in consistent positions over all reconstructions, except for a few cases when individual VIP reconstructions dropped taxa, which shifted the barrier in one direction towards one of the included groups but never changed its directionality or general position.

Biogeographic analyses indicate few putative long-range dispersal events between different European mountain systems within the *I. hellwigii* and *I. manicata* groups. The Iberian-Alpine group showed a general pattern of diversification coinciding with the division of ancestral areas, resulting in decreasing distribution areas with decreasing phylogenetic age of the clades, with evidence for long-range dispersal generally absent. Thus geographic proximity generally coincides with closer phylogenetic relationship within the Iberian-Alpine group (Fig. 6). Species distributions within the Iberian-Alpine

group are not strictly allopatric but overlap in many cases, and in the Iberian distributional area at least two sympatric sister species are present.

DISCUSSION

Higher-level phylogeny.—Comparing phylogenetic hypotheses (Fig. 2) of Martens (1969b) and Dresco (1970) with molecular-based concatenated (Fig. 3) or *BEAST trees (Fig. 4) reveals little congruence. These alternative hypotheses agree only in the separate (Martens 1969b; Dresco 1970) and basal placement (Martens 1969b) of the *I. hellwigii* group. Further similarities only partly coincide with para- and polyphyletic groups, except for Hadži's *Odontopalpa*, that receives weak support in our analyses. Hadži (1931) included the species *I. kollari* (sub *I. triglavensis*) and *I. dentipalpis* (presently split into *I. lithoclasica* and *I. dentipalpis* sensu stricto; Schönhofer & Martens 2010a) in this subgenus and assumed *I. ravasinii* to be included, although he had no males to confirm this placement. Martens (1969b) considered the genus *Ischyropsalis* too small to justify establishment of subgenera. Accepting *Odontopalpa* would require the definition of several additional subgenera, which he felt carried little taxonomic value—we generally agree with this recommendation.

While the detailed studies of previous authors refined the characters for species-level taxonomy, higher-level groups (as implied by molecular phylogeny) now lack morphologically diagnostic characters. This situation is similar to other harvestmen groups, for example *Trogulus* Latreille 1802, where characters delineating species appear clearer than those characterizing higher-level groups (Schönhofer & Martens 2010b). The molecular results for *Ischyropsalis* suggest that most of the presently used morphological systematic characters are randomly distributed throughout the phylogeny. Even male-specific characters of the genitalia and cheliceral apophyses seem problematic for use as phylogenetic characters. This is not completely unexpected as Martens (1969b) mentioned the relatively simple organization of the penis in *Ischyropsalis*, perhaps prone to homoplasy or morphological stasis.

Character conflict is well-illustrated by the "subspecies" of *I. pyrenaea*, previously believed to be closely related based on morphology, but apparently belonging to different groups within *Ischyropsalis* (Fig. 3). The proposed shared characters of the *I. pyrenaea* subspecies (Martens 1969b, 1978), including cheliceral spination and apophyses, the male cheliceral bristle field and the overall similarity of the male genital morphology (e.g., un-lobed and keeled sclerites of the penial glans), are likely convergent. Indeed, the structure of these sclerites differs considerably in detail, particularly in the connection towards the sclerotised part of the penial stylus (Fig. 1). In *I. pyrenaea pyrenaea* this sclerite is rather broad, while in *I. pyrenaea alpinula* it is extremely narrow, appearing almost petiolate.

An overview of characters for diagnosing recovered molecular clades is given below. *Ischyropsalis hadzii* Roewer 1950, *I. gigantea* Dresco 1968, *I. petiginosa* Simon 1913 and *I. cantabrica* Luque & Labrada 2012, not included in our data set, are also assigned tentatively to these larger groups.

    1) *I. hellwigii* group (two described subspecies *I. h. hellwigii*, *I. h. lucantei* Simon 1879): Chelicerae massive, males with
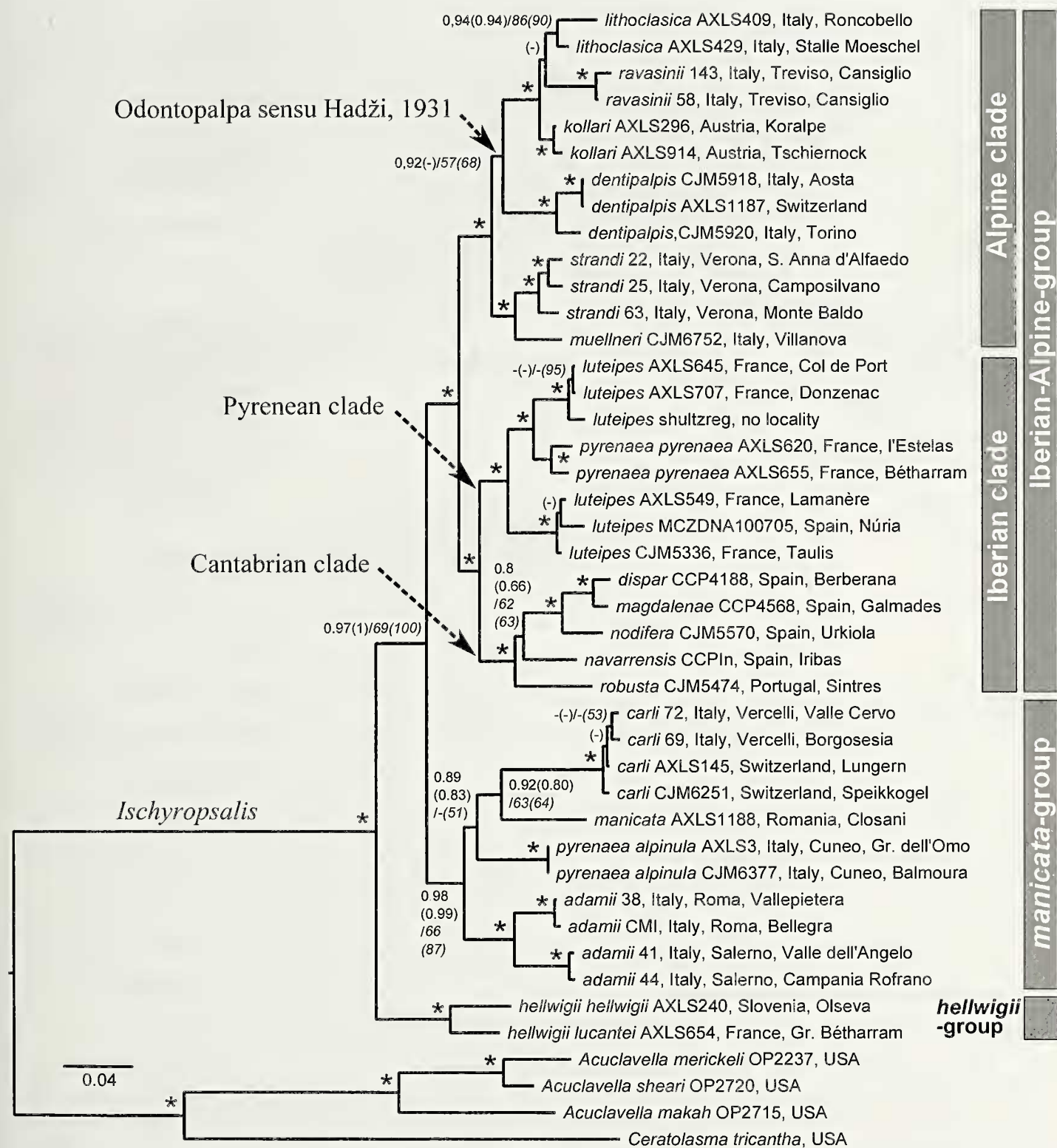
Figure 3.—Bayesian consensus phylogram based on concatenated molecular data. Numbers at nodes show support values resulting from different analyses: 1) Bayesian posterior probabilities; numbers in parentheses are Bayesian posterior probabilities after removal of outgroup genera and realignment of 28S and EF1α intron, 2) maximum likelihood bootstrap values (in italics); in parentheses after removal of outgroup and realignment of 28S and EF1α intron. Nodes with pp = 1.00 and bootstrap values >94 indicated by asterisks. Support values below 0.94 pp and 70 bootstrap not shown.

Figure 4.—*BEAST species tree. Basal nodes graphically shortened to allow expanded view of ingroup. Posterior probabilities given at nodes; pp of 1.00 indicated by an asterisk. Distribution of species group taxa according to Martens (1969b) is symbolized at tips: *dentipalpis* group indicated by black squares, *kollari* group indicated by open diamonds. *I. lithoclasica* was formerly considered *I. dentipalpis*, *I. dentipalpis* was considered *I. helvetica* (Martens 1969b; 1978), and *I. pyrenaea alpinula* was described as a subspecies by Martens (1978). Predominantly cave-dwelling species indicated by a cave icon.

a large dorsal cheliceral glandular bristle field. Sclerite of penial gland small, with irregularly spaced small spines. *Ischyropsalis h. lucantei* is restricted to the central Pyrenees, while *I. h. hellwigii* occupies a large area from Germany to Southern Poland.

The following groups share long bristles on the penial glandes:

II) *I. manicata* group (*I. adamii, I. carli, I. manicata, I. pyrenaea alpinula*): Penial sclerites narrow-stalked, appearing almost narrow leaf- to broad heart-shaped.
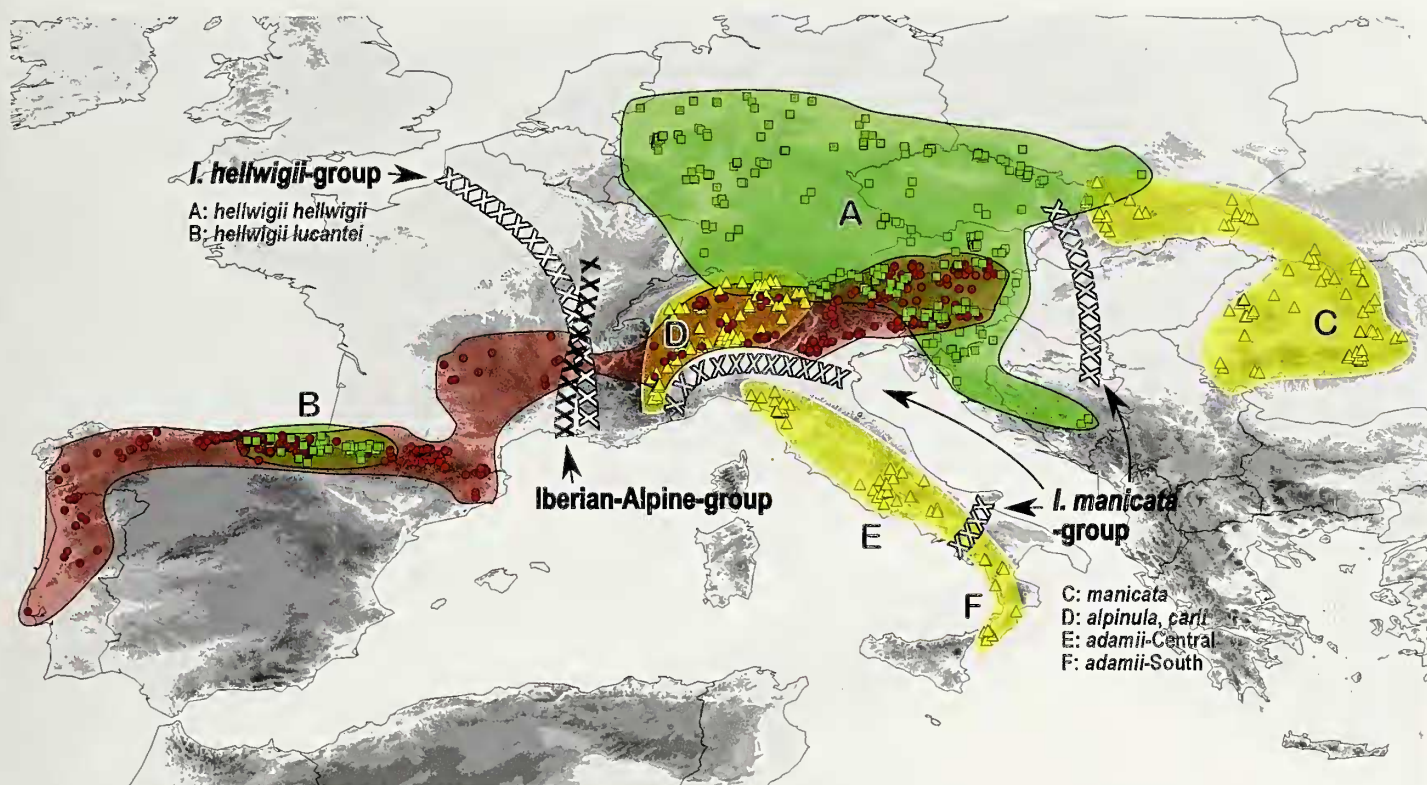
Figure 5.—Distribution of primary *Ischyropsalis* groups in Europe, with VIP barriers shown as lines of crosses. Major groups include the *I. hellwigii* (green squares), *I. manicata* (yellow triangles), and Iberian-Alpine (red circles) groups.

Sclerites always keeled and never bilobed, males with small distinctly circumscribed distolateral cheliceral glandular bristle field. Individual species have a rather widespread distribution. Two species in the western Alps (*I. carli*, *I. pyrenaea alpinula*), and one species in the Carpathians (*I. manicata*) and Apennines (*I. adamii*), respectively; in the latter two areas these represent the only *Ischyropsalis* taxa known.

III) Iberian-Alpine group: Including the remaining species. Penial sclerites broad-stalked or not narrowed towards intersection with stylus. Otherwise morphologically heterogeneous and best separated by geographic distribution.

IIIa) Alpine-clade: the group is separated into two morphologically separable clades that share few characteristics, maybe the bilobed penial sclerite, which is not split in *I. dentipalpis*. Most species of the first group (*I. dentipalpis*, *I. kollari*, *I. lithoclasica*, *I. muelheri* Hamann 1898) exhibit a unique patellar apophysis on the male palp (missing in *I. hadzii* that likely belongs to this group as well) and have a large plateau-like bristle-field with sparse and scattered setation on the basal cheliceral segment (also in *I. hadzii*). Members of the second group within this clade (*I. ravasinii* and *I. strandi* Kratochvíl 1936) lack the palpal apophyses and bristle field and apophyses are reduced or missing. Both groups are distributed mainly in the Central to Eastern Alps, with only *I. dentipalpis* in the Western Alps.

IIIb) Iberian-clade (including two subclades): many species have keeled penial sclerites, otherwise sclerites are always deeply bi-lobed. Males have large triangular dorsodistal cheliceral apophyses. Members of the Pyrenean clade (*I. lutcipes* [two lineages], *I. pyrenaea pyrenaea*) share no perceivable characters, which highlights that most observed character states can evolve rapidly. Uniting features include distribution in the Pyrenees and molecular synapomorphies. The Cantabrian clade (*I. dispar* Simon 1872, *I. magdalenae* Simon 1881, *I. navarrensis*, *I. nodifera*, *I. robusta*, *I. petiginosa*, likely *I. cantabrica* and *I. gigantea*) has a primary uniting character, the bristle field on the basal cheliceral segment, situated on the distomedial side. The cheliceral apophysis is generally very large, often triangular and pointed, viewed laterally. This group is in need of further evaluation, including study of more populations, missing species and more refined geographic analyses.

Species-level systematics.—Our molecular phylogenetic results fail to recover two described species as monophyletic, or recover species as monophyletic but with high internal genetic divergence, perhaps consistent with cryptic or incipient speciation. As previously discussed the two subspecies of *Ischyropsalis pyrenaea* are distantly related (Figs. 3 & 4). Considering their phylogenetic distance, they are hereby formally elevated to species (*I. pyrenaea pyrenaea* to *I. pyrenaea* and *I. pyrenaea alpinula* to *I. alpinula*). A careful redescription is warranted and may reveal further phyloge-
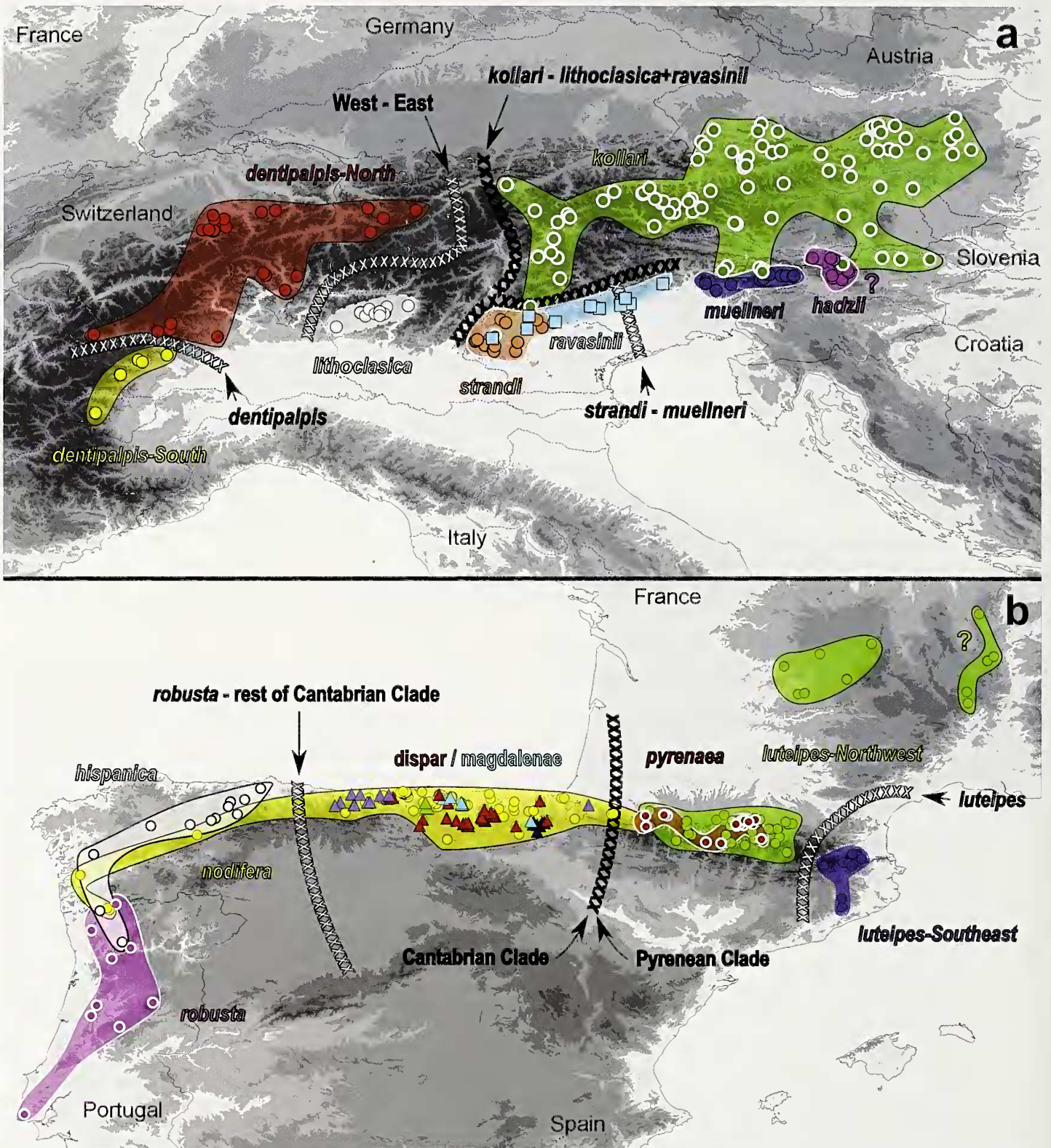
Figure 6.—a. Distribution of the *Ischyropsalis* Alpine clade in the Alps showing biogeographic reconstructions within major clades; b. Distribution map of the *Ischyropsalis* Iberian clade on the northern Iberian Peninsula and southern France. The distribution of microendemic species in the Cantabrian Mountains of northern Spain was not further resolved. For both a & b, colors correspond to individual species and lineages as supported by *BEAST and other analyses, and VIP barriers are shown as lines of crosses. Question marks indicate populations or species where phylogenetic information is lacking.

netically useful characters. Also in need of further study is the paraphyletic *I. luteipes*, with the morphologically different *I. pyrenaea* nested within this lineage (Fig. 3). Dresco (1970) discussed three "ecoforms" of *I. luteipes* but did not formally describe these or provide characters for delineation. Character reinvestigation and additional comprehensive geographic sampling is required.

The central and southern lineages of the Italian *Ischyropsalis adamii* are genetically clearly separable (Fig. 3), while material from the isolated northern population was not available for this study. Individual names for all three populations have been proposed by earlier authors and require reassessment (southern Italy: *I. adamii*; northern Italy: *I. apuanus* Caporiacco 1930; central Italy: *I. apuanus nanus* Dresco 1968). Similarly, our data indicate that the southwestern-most population of *I. dentipalpis* is genetically distinct. This species has been recently re-evaluated based on morphology (Schönhofer & Martens 2010a), and further studies of its fragmented distribution were suggested. Our results indicate differentiation within *I. dentipalpis* but refute a close relationship to *I. lithoclasica* as hypothesized by Schönhofer and Martens (2010a).

Historical biogeography.—While Martens (1969b) assumed widespread species groups with strictly allopatric species, our phylogenetic results indicate a clearly different pattern. Herein, diversification and speciation is generally restricted to the same mountain range, specifically the Alps, the Cantabrian Mountains, the Pyrenees, and, to a lesser extent, the Apennines. Only in the monotypic *Ischyropsalis hellwigii* and *I. manicata* groups are species and lineages distantly allopatric, consistent with old vicariance or long-range dispersal (Fig. 5). The remaining *Ischyropsalis* species belonging to the Iberian-Alpine group are frequently sympatric, sometimes with completely overlapping distributions of closely related taxa, e.g., *I. luteipes* and *I. pyrenaea*. The biogeographic pattern within the Iberian-Alpine group shows a general trend of decreasing distribution area with decreasing taxonomic level, with the distribution of descendants contained within the distributional area of reconstructed ancestors. Therefore, dispersal seems not to be the main cause of diversification within *Ischyropsalis*, which remains difficult to test without reliably assignable fossils. The lack of *Ischyropsalis* fossils also means that we cannot rule out the presence of now extinct representatives in areas outside the known distribution of the genus.

Evolution of habitat preferences.—Most extant *Ischyropsalis* species are confined to montane habitats, preferring microhabitats with moderate to cold temperatures and high and constant humidity. On the Iberian Peninsula *Ischyropsalis* species are confined to mountain regions situated along the northern and western coast, characterised by high and consistent rainfall and moderate variation in annual temperatures (Immerzeel et al. 2009). Precipitation in the Alps is generally high, and low temperature microhabitats are abundant at high altitudes in this mountain chain.

It is noticeable that *Ischyropsalis* shows a general trend from more generalist and widespread species (*I. hellwigii* and *I. manicata* groups) towards specialized short-range endemics (most members of the Iberian-Alpine group). This trend manifests in the high number of cave endemics in *Ischyr-*

opsalis, which may be considered extreme ecological specialists. Molecular phylogenetic trees include no large clades of exclusively cave-dwelling taxa (Fig. 4). Rather, it appears that cave species have evolved multiple times in clades of mainly epigean species, a pattern reported from many other cave radiations (Porter 2007), including harvestmen (Derkarabetian et al. 2010; Hedin & Thomas 2010; Derkarabetian & Hedin 2014). However, the degree of cave specialization in *Ischyropsalis* is variable, with many surface-dwelling species regularly entering caves. Caves are the only available habitats at low altitudes, with surface habitats such as alpine gravel or forests increasing towards high altitudes. Fully troglomorphic taxa are rare, as most species retain eyes. The tendency towards troglomorphism correlates with a preference for cooler and more stable temperatures. For example, temperature preferences for the surface-dwelling *I. luteipes* were recorded at 11.5 °C with a range of 10 °C (Juberthie 1964). In the troglomorphic *I. pyrenaea pyrenaea* the mean temperature is comparable, but the range does not exceed 5 °C. A similar preference range is exhibited by eastern Alpine cave endemics but with mean preferences shifted to even lower temperatures (*I. strandi*: 3.5–7.2 °C; *I. muellneri*: 5.2–7.8 °C; Juberthie 1964). The frequent evolution of cave specialization enables the regional co-occurrence of epigean and predominantly troglomorphic species. The preference for low temperatures might also explain the general absence of *Ischyropsalis* in more southern cave systems as on the Balkan, as these caves generally show higher mean temperatures.

Members of the Iberian-Alpine group and all cave endemic species may be vulnerable to climate change given their geographically restricted ranges and narrow ecological preferences. Considering these factors, it will be important to protect relevant cave habitats as comprehensively as possible.

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Appendix.—Specimens included in phylogenetic analyses.

Organism	Voucher	Locality	Lat (N)	Long (E)	28S acc.-no.	COI acc.-no.	EF1a acc.-no.
Acuclavella makah Richart and Hedin 2013	OP2715	USA: Washington, Clallam Co., Hoko Falls	no data	no data	KF181753[1]	KF181737[1]	KF181770[1]
Acuclavella merickeli Shear 1986	OP2237	USA: Idaho, Idaho Co., FS 443 0.8 mi S of Selway Rv Rd	no data	no data	KF181751[1]	KF181733[1]	KF181767[1]
Acuclavella sheari Richart and Hedin 2013	OP2720	USA: Idaho, Idaho Co, FS 592	no data	no data	KF181756[1]	KF181743[1]	KF181777[1]
Ceratolasma tricantha Goodnight and Goodnight 1942	OP989	USA: OR, Curry Co., E. Gold Beach (COI sequence from different specimen[2])	no data	no data	JX573543[2]	GQ912865[3]	JX573601[2]
Ischyropsalis adamii Canestrini 1873	FEM 038/G/2006	Italy: Lazio, Prov. Roma, Vallepietera, Pozzo Cornetto	41.94	13.23	KP224348	KP224375	KP224405
Ischyropsalis adamii Canestrini 1873	Coll. M. Isaia (CMI) I_adamii	Italy: Lazio, Roma, (GPS for) Bellegra, Grotta dell'Arco 5La	41.88	13.03	-	KP224376	KP224406
Ischyropsalis adamii Canestrini 1873	FEM 041/G/2006	Italy: Campania, Salerno, Valle dell'Angelo, Grotta dei Briganti	40.29	15.39	KP224349	KP224377	KP224407
Ischyropsalis adamii Canestrini 1873	FEM 044/G/2006	Italy: Campania, Salerno, Campania Rofrano, Grava di Carlo (del pian delle lepri)	40.21	15.42	KP224350	KP224378	KP224408
Ischyropsalis carli Lessert 1905	FEM 072/G/2006	Italy: Piemonte, Prov. Vercelli, Cavità artificiale alta valle Cervo ex miniera alpe Machetto	45.61	8.04	KP224351	KP224379	KP224409
Ischyropsalis carli Lessert 1905	FEM 069/G/2006	Italy: Piemonte, Prov. Vercelli, Valduggia, Comune di Borgosesia, Grotta delle Arenarie (2509 Pi/VC)	45.714	8.315	-	KP224380	KP224410
Ischyropsalis carli Lessert 1905	AXLS 145	Switzerland: Kanton Oberwalden, Lungern, Wengenhöhle (661245/186818)	46.83	8.24	JX573544[2]	JX573638[2]	JX573602[2]
Ischyropsalis carli Lessert 1905	CJM 6251	Switzerland: Kanton Bern, stream above Gantrischseeli (lake N mountain Gantrisch)	46.70307	7.45150	KP224352	-	-
Ischyropsalis dentipalpis Canestrini 1872	AXLS 1187	Switzerland: Kanton Obwalden, Sachseln, Sachseler Seefeld, Höhle S1 (Swiss-Coordinates 659710 / 182750)	46.793	8.225	-	KP224381	KP224411
Ischyropsalis dentipalpis Canestrini 1872	CJM 5918	Italy: Prov. Aosta, Gressoney, Pozzo A di Punta Jolanda ((Ao/AO 2075)	45.84	7.84	KP224353	KP224382	-
Ischyropsalis dentipalpis Canestrini 1872	CJM 5920	Italy: Piemonte, Prov. Torino, Brosso, Valchiusella-Valley, Buca del Ghiaccio della Cavallaria (Pi/TO 1609)	45.52	7.80	KP224354	KP224383	-
Ischyropsalis dispar Simon 1872	CCP CCP4188	Spain: Burgos, t.m. Villalba de Losa, (Perilde) Berberana, Sierra Salvada, Cueva Albia (VN9-5-)	42.9706	-3.0728	KP224355	KP224384	KP224412
Ischyropsalis helwigii helwigii (Panzer 1794)	AXLS 240	Slovenia: Olševa-Mountains, locality Sadni Travniki	46.45708	14.68605	JX573545[2]	JX573639[2]	JX573603[2]
Ischyropsalis helwigii lucantei Simon 1879	AXLS 654	France: Midi-Pyrénées, Dép. Hautes-Pyrénées, Lourdes, Saint-Pé-de-Bigorre, Grottes de Bétharram	43.10234	-0.18478	KP224356	KP224385	KP224413
Ischyropsalis kollari C.L. Koch 1839	AXLS 296	Austria: Steiermark, Koralpe, Wolfsberg, Großer Speikkogel, locality Erlenloch	46.79874	14.95092	JX573546[2]	-	JX573604[2]
Ischyropsalis kollari C.L. Koch 1839	AXLS 914	Austria: Kärnten, Treffling, Tschiernock Mt.	46.85279	13.58791	KP224357	KP224386	KP224414

Appendix.—Continued.

Organism	Voucher	Locality	Lat (N)	Long (E)	28S acc.-no.	COI acc.-no.	EF1a acc.-no.
Ischyropsalis lithoclasica Schönhofer and Martens 2010	AXLS 429	Italy: Prov. Bergamo, Valle Valzurio, Oltressenda Alta, picknick-area W Stalle Möschel	45.93810	10.00446	KP224358	-	KP224415
Ischyropsalis lithoclasica Schönhofer and Martens 2010	AXLS 409	Italy: Prov. Bergamo, Roncobello, Corno Branchino and Lago Branchino	45.94931	9.80257	KP224359	KP224387	KP224416
Ischyropsalis luteipes Simon 1872	AXLS 645	France: Midi-Pyrénées, Dép. Ariège, Col de Port	42.89675	1.44922	KP224360	KP224388	KP224417
Ischyropsalis luteipes Simon 1872	AXLS 707	France: Limousin, Dép. Corrèze, Brive-la-Gaillarde, N Donzenac	45.23460	1.54536	KP224361	KP224389	-
Ischyropsalis luteipes Simon 1872	no data	no data	no data	no data	-	-	AF240870[4]
Ischyropsalis luteipes Simon 1872	Museum of Comparative Zoology 100705	Spain: Girona, Queralps, Núria, Font de l'Home Mort	42.36	2.12	GQ912767[3]	-	-
Ischyropsalis luteipes Simon 1872	CJM 5336	France: Languedoc-Roussillon, Dép. Pyrénées-Orientales, D618 between St.-Marsal und Taulis	42.52925	2.6112	GQ466293[5]	KP224390	KP224418
Ischyropsalis luteipes Simon 1872	AXLS 549	France: Languedoc-Roussillon, Dép. Pyrénées-Orientales, Lamanère	42.35874	2.51685	KP224362	KP224391	KP224419
Ischyropsalis magdalenae Simon 1881	CCP CCP4586	Spain: País Vasco, Biscay, Galmades, Mina la Fragua			KP224363	KP224392	KP224420
Ischyropsalis manicata C.L. Koch 1865	AXLS 1188	Romania: Closani, Ranca, cave	45.08	22.80	-	KP224393	-
Ischyropsalis muellneri Hamann 1898	CJM 6752	Italy: Friuli, Prov. Udine, Luzevera, Villanova, Fr 548, Abisso Mario Grassi	46.25	13.27	KP224364	KP224394	-
Ischyropsalis navarrensis Roewer 1950	CCP CCP I.n.	Spain: Navarra, Sierra de Aralar, Iribas, Sima de Lezegalde	42.986	-1.904	KP224365	KP224395	KP224421
Ischyropsalis nodifera Simon 1879	CJM 5570	Spain: País Vasco, Prov. Álava, ca. 5km S of pass Urkiola	43.0734	-2.6619	KP224366	KP224396	-
Ischyropsalis pyrenaea alpinula Martens 1978	AXLS 3	Italy: Piemonte, Prov. Cuneo, Grotta O-5 dell'Omo	44.3722	7.1374	KP224367	KP224397	KP224422
Ischyropsalis pyrenaea alpinula Martens 1978	CJM 6377	Italy: Piemonte, Prov. Cuneo, betw. Grange Pieccia and Chiappi, Grotta Balmoura	44.4497	7.1502	KP224368	KP224398	-
Ischyropsalis pyrenaea pyrenaea Simon 1872	AXLS 620	France: Midi-Pyrénées, Dép. Ariège, NW Cazavet, Grotte de l'Estelas	42.99665	1.0101	JX573547[2]	JX573641[2]	JX573605[2]
Ischyropsalis pyrenaea pyrenaea Simon 1872	AXLS 655	France: Midi-Pyrénées, Dép. Hautes-Pyrénées, Lourdes, Saint-Pé-de-Bigorre, Grottes de Bétharram	43.10234	-0.18478	KP224369	KP224399	KP224423
Ischyropsalis ravasinii Hadži 1942	FEM 143/G/2006	Italy: Veneto, Prov. Treviso, Pian del Cansiglio, Bus de la Genziana	46.028	12.407	KP224370	KP224400	KP224424
Ischyropsalis ravasinii Hadži 1942	FEM 058/G/2006	Italy: Veneto, Prov. Treviso, Pian del Cansiglio, Bus de la Genziana	46.028	12.407	KP224371	-	KP224425
Ischyropsalis robusta Simon 1872	CJM 5474	Portugal: 20 km W Lissabon, Sintres	38.79325	-9.3929	GQ466294[5]	KP224401	KP224426
Ischyropsalis strandi Kratochvíl 1936	FEM 022/G/2006	Italy: Veneto, Prov. Verona, S. Anna d'Alfaedo, Spluga della Preta	45.678	10.952	KP224372	KP224402	KP224427
Ischyropsalis strandi Kratochvíl 1936	FEM 025/G/2006	Italy: Veneto, Prov. Verona, Camposilvano, Buso dell'Arena	45.62	11.03	KP224373	KP224403	KP224428
Ischyropsalis strandi Kratochvíl 1936	FEM 063/G/2006	Italy: Veneto, Prov. Verona, Monte Baldo, Novezzina Grotta RH9	45.69	10.85	KP224374	KP224404	KP224429

[1] Richart & Hedin 2013; [2]Schönhofer et al. 2013; [3]Giribet et al. 2010; [4]Shultz & Regier 2001; [5]Schönhofer & Martens 2010b.